

**BIOMATERIALS FOR THE CENTRAL NERVOUS SYSTEM**

Contract No. NO1-NS-1-2338

Quarterly Progress Report #13

January 31, 2005

The University of Michigan and  
The University of Utah

David C. Martin and Patrick A. Tresco

**Quarterly Progress to:** National Institute of Health  
**Contract Monitor:** Joseph Pancrazio, Ph.D.  
**Research Contract** "Biomaterials for the Central Nervous System"  
**Contract No.** NO1-NS-1-2338  
**Principal Investigators:** David C. Martin and Patrick A. Tresco  
**Date:** January 31, 2005

## **Overview**

This report is a summary of our activity in the twelfth quarter of our contract, corresponding to the period from October 31, 2004 to January 31, 2005. We include information about the in-vivo performance of hydrogel coated probes, which showed the sensitivity of coating thickness in determining the quality of the recording, and the fact that the size of the cells was not as important as their proximity to the electrode surface. We also describe our nanoindentation results that confirmed the relationship between the compliance of the polymer coating and their electrical transport properties. We also report on the delivery of anti-inflammatory agents from electrospun nanofibers. Finally, we also describe the electrochemical polymerization of PEDOT in the presence of poly(l-lysine) (PLL), a polymer known to promote cell adhesion.

## Functional evaluation of hydrogel coated electrodes

In the previous report, we investigated the affect of hydrogel coatings on the probes and found that the recording quality of the hydrogel coated electrode can be affected significantly even with very thin coatings. Therefore, in this report, we are intensively investigating the relation between alginate coating and recording quality and also we are trying to develop technique to overcome that problem.

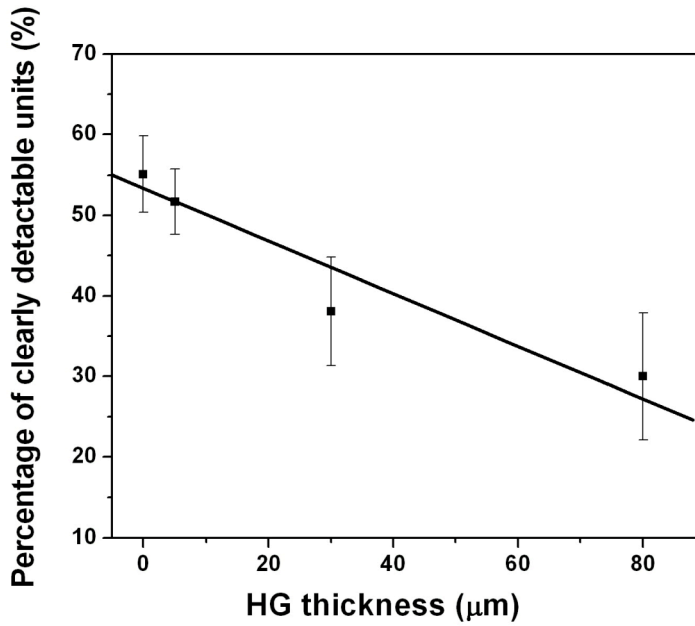
## Experimental

### 1. Recording quality of hydrogel coated electrode

Experimental details were described in a previous report (NIH quarterly report 11). Briefly, neural recordings were collected using Plexon system, and the SNRs were categorized into 3 schemes: high ( $\text{SNR} > 4.0$ ), medium ( $4.0 > \text{SNR} > 3.5$ ) and low ( $\text{SNR} < 3.5$ ) SNR using custom software developed at the CNCT. The data set includes at least 40 individual recording sites. Acute experiments in guinea pig were used to verify and characterize *in vivo* electrical functionality of the hydrogel itself. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1085) have been observed.

#### 1-1 In the Auditory cortex : 200 ms noise burst

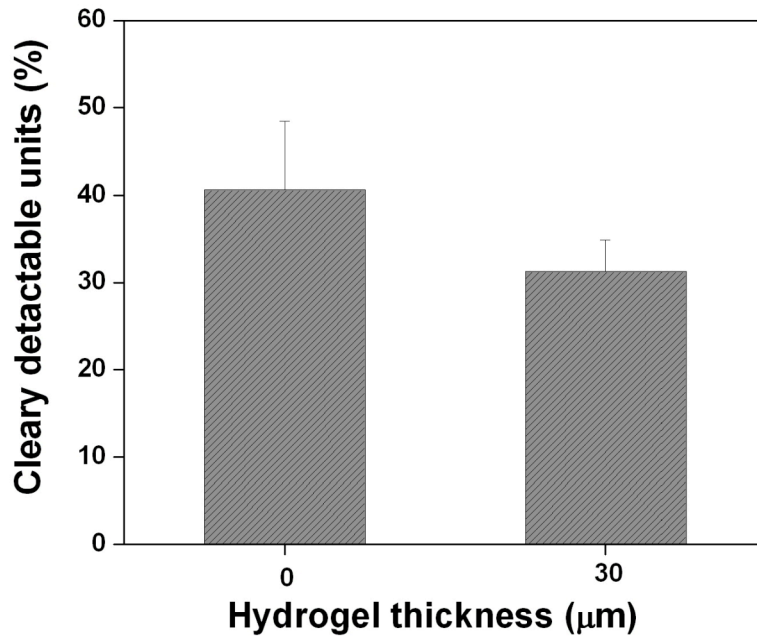
In this experiment, the typical extracellular potential recorded on an electrode had unit amplitudes ranging from approximately 50  $\mu\text{V}$  to 300  $\mu\text{V}$  with a SNR of 3.27 to 8.01. Figure 1 shows the percentage of clearly detectable units decreases as a function of the thickness of the hydrogel coatings. In this data, the functional loss of opened cortex over time was considered carefully to get more accurate data. An average of clear units on bare electrodes exhibited more than 4.0 SNR was 56 % which is smaller than the result from the previous reports from motor cortex ( $79 \pm 9$  %, *Justin Williams et al, Long-term neural recording characteristics of wire electrode arrays implanted in cerebral cortex, Brain Reserch Protocols, 4, 303-313, 1999* and 90 %, *Rio Vetter et al, Chronic neural recording using silicon-substrate Microelectrode arrays implanted in cerebral cortex. 51, 896-904, 2004*). Note that the decrease of number of clear units happens even in the case of the thinnest coating (5  $\mu\text{m}$ ). The number of clearly detectable units then gradually becomes smaller as a function of thickness of the hydrogel coating indicating that the affect of hydrogel can become more serious with relatively thick coatings, and with 80  $\mu\text{m}$  thick coating only 30% of the electrode sites can record clear signals from the neurons around the probe. This is consistent with the result of spatial distribution of neurons related to recording quality: it is hard to record a clear signal if the distance between the electrode sites and firing neurons exceed 100  $\mu\text{m}$ . We think this affect is due to the fact that the reswelling of the dehydrated hydrogel coatings in the brain after implantation pushes the active neurons away from the probe surface. We do not believe this is related to a decrease in signal transport through the hydrogel, since we have shown in previous progress reports that the measured impedance of the electrode is not significantly changed even for relatively thick (up to 100  $\mu\text{m}$ ) hydrogel coatings. These results clearly indicate the importance of the local proximity of cells to the electrode surface for maintaining high quality recordings.



*Figure 1. The number of clearly detectable units as a function of the thickness of hydrogel coatings in the auditory cortex with 200 ms noise burst.*

## **1-2 In the Cerebellum: spontaneous firing**

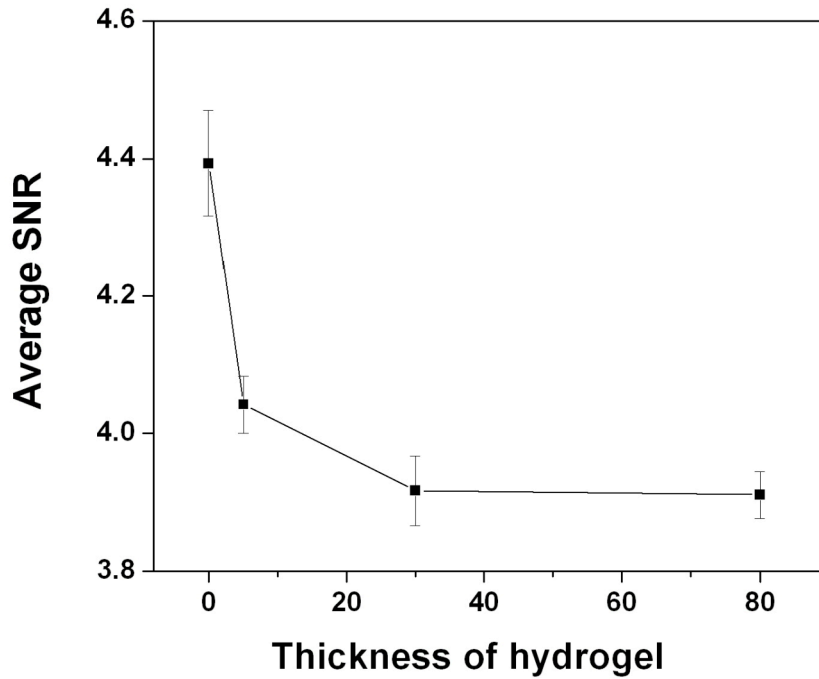
In order to investigate the relation between the size of neurons and the proximity, the electrodes were implanted into the cerebellum which has larger neurons than the auditory cortex (Figure 2). Without stimulation, about 42% of electrode sites can receive clear signals and it dropped down to about 28% with a 30 μm hydrogel coating applied. We think this is because we were recording spontaneous firing from the cerebellum without external stimulation. In this experiment, we did not find any significant difference from the case of the auditory cortex besides the population of clear units was slightly smaller than auditory cortex. These results indicate that the local proximity of cells to the electrode sites is more critical than the size of the neurons for maintaining high quality recordings.



*Figure 2. The number of clearly detectable units with and without hydrogel coatings in the cerebellum. Despite larger neurons present in the cerebellum, there is no increase in the percentage of clearly detectable units.*

### 1-3 SNR of clearly detectable units

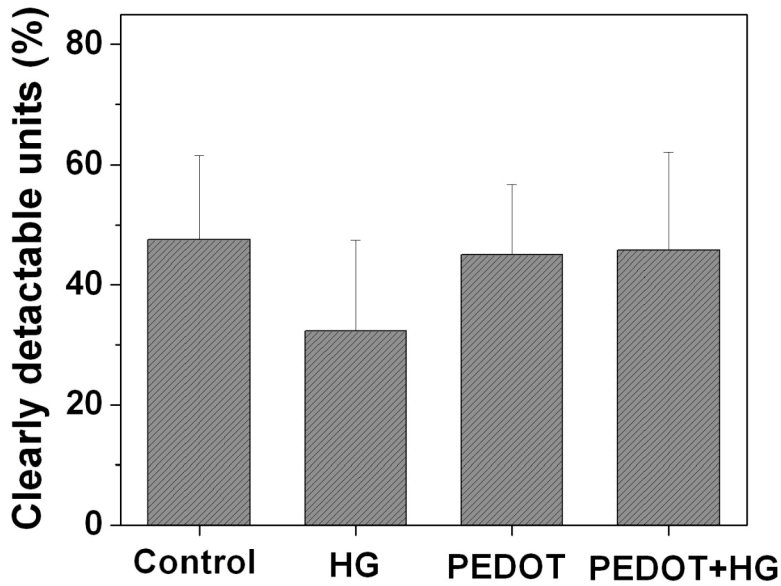
Figure 3. shows the trend in signal quality, as indicated by SNR, as a function of hydrogel thickness. Note that the decrease of SNR as a function of hydrogel coating happens dramatically even with 5 μm thick hydrogel coatings indicating that the signal quality can be affected more dramatically by applying hydrogel coating compared with number of clear units and the loss of signal quality is not linear with the hydrogel thickness. The drop of the average SNR levels off quickly as hydrogel coating gets thicker (>30 μm) showing that the SNR at 30 μm thick hydrogel was almost same to the 80 μm. It is interesting to observe that the average SNR didn't decrease linearly to the hydrogel thickness while the number of clear units did.



*Figure 3. Average SNR on clearly detectable units as a function of hydrogel coating.*

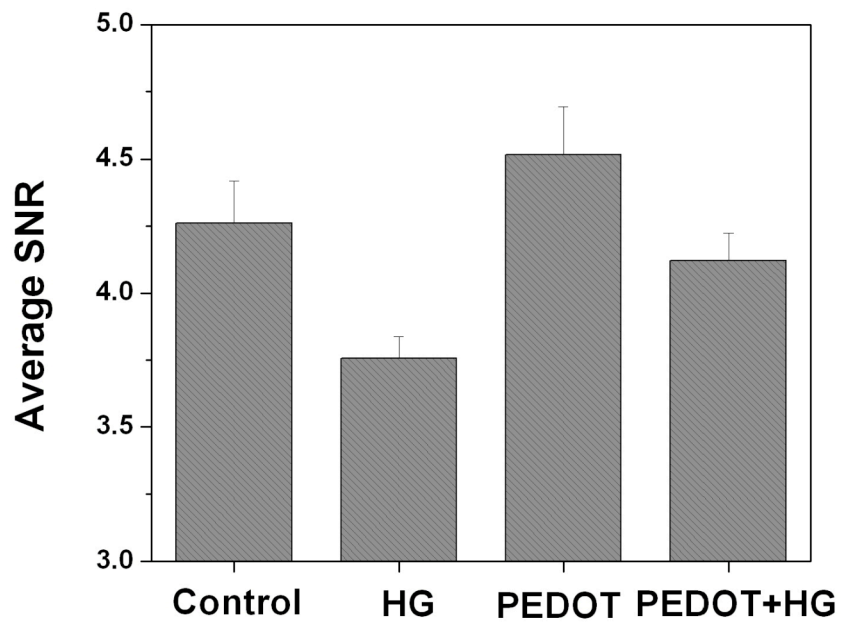
## **2. Recording quality of modified electrode using PEDOT**

We investigated if the loss of recording quality including decrease of number of clear units and the drop of SNR caused from applying hydrogel coating can be stored back using conducting polymer on the electrode site. PEDOT (Poly(3,4-ethylenedioxythiophene)) was deposited on the electrode site under galvanostatic conditions at room temperature using 6 nA for 10 min. In general, PEDOT deposition can reduce the impedance and facilitate electrical transportation. However PEDOT deposited electrode site did not show increase of the number of clear units. We think that is because conducting polymer has no influence in the increase of the number of neurons within effective distance sufficient to receive clear signal. It is however obvious that PEDOT deposition does support hydrogel coated electrodes to recover the number of clear units as shown the clear units of HG+PEDOT electrodes are almost same to the bare electrodes (Figure 3).



*Figure 4. The number of clearly detectable units with various different condition including control, hydrogel coating on the probe, PEDOT deposition on the electrode sites, PEDOT deposition on the electrode sites under the HG coatings.*

Figure 4. shows the average SNR of the electrodes with or without modification. Note that the average SNR of PEDOT deposited electrode shows smaller than bare electrode. We are currently trying to investigate this unexpected result. PEDOT deposition on the electrode sites under hydrogel coatings partially recovered the SNR of the electrodes, yet the SNR of the PEDOT+HG was still not as high as the bare electrodes.



*Figure 5. Average SNR on clearly detectable units with various different condition including control, hydrogel coating on the probe, PEDOT deposition on the electrode sites, PEDOT deposition on the electrode sites under the HG coatings.*



## ***Correlations of Electrical and Mechanical Properties of Conducting PEDOT Coatings on Neural Probes***

### **Overview:**

It has been observed that the modulus of silicon is about 170 GPa, while a value of 0.1 MPa for the modulus of human brain was obtained [1]. So it is necessary to accommodate the large differences in mechanical properties between the rigid silicon-based device and the soft tissue of the brain. Recently we have found that soft, low impedance, and biologically active conducting PEDOT coatings can be prepared by the electrochemical deposition on the electrode sites [2-3]. By correlating measurements of probe electrical properties with surface morphologies we have found that maximizing the effective surface area of the electrode coating makes it possible to minimize the electrical impedance. Nanoindentation is a very useful technique and is particularly suited to thin films, micron- or nano-sized area applications, and it has been used to generate information about the deformation behavior of a number of polymer thin films, coatings, blends and composites [4-5].

In this quarterly report, we will evaluate the mechanical properties of conducting polymer PEDOT coatings and compare them to their electrical properties.

### **Experimental:**

The fabrication and the electrical properties characterization of PEDOT coatings on the electrode sites have been extensively investigated in our previous papers and progress reports [2-3]. The mechanical properties of PEDOT coatings were characterized using nanoindentation (Nano instruments Nano II). A pyramidal-shaped diamond tip (Berkovitch tip) is progressively pressed onto the same surface by  $\mu\text{N}$  scale force. A quartz standard sample was used to calibrate the instrument and calculate the tip area function. Information about the sample surface topography was obtained by AFM.

### **Results and Discussion:**

Fig.1a shows typical load-displacement curves for a variety of PEDOT coatings (with different deposition charge) on 8-site electrode probe. The two main steps, loading cycle, and unloading cycle are noted in these figures. There are three important parameters in these figures: maximum load ( $P_{\text{max}}$ ), maximum displacement ( $h_{\text{max}}$ ), and plastic deformation, ( $h_p$ ). For example, for the PEDOT coated electrode site with passing through deposition charge of  $3.6 \mu\text{C}$  (or 10 min), the  $P_{\text{max}}$ ,  $h_{\text{max}}$  and  $h_p$  are 0.92 mN, 548 nm and 512 nm, respectively. The AFM image of this indent is shown in Fig.1b. The triangular shape left by the Berkovich indenter can be clearly seen. The section analysis under the image illustrates an indentation depth of 520 nm, which is consistent with the plastic deformation data ( $h_p$ ) mentioned above (512 nm). The maximum displacement for different PEDOT coated sites with having a different coating thickness was in the range of 900 nm, while only 134 nm for gold-coated silicon site under the same load. These load vs. displacement curves illustrate the softer nature of conducting polymer, compared with the

harder silicon devices. The softer conducting polymer surface requires lower normal loads to induce a comparable indenter penetration.

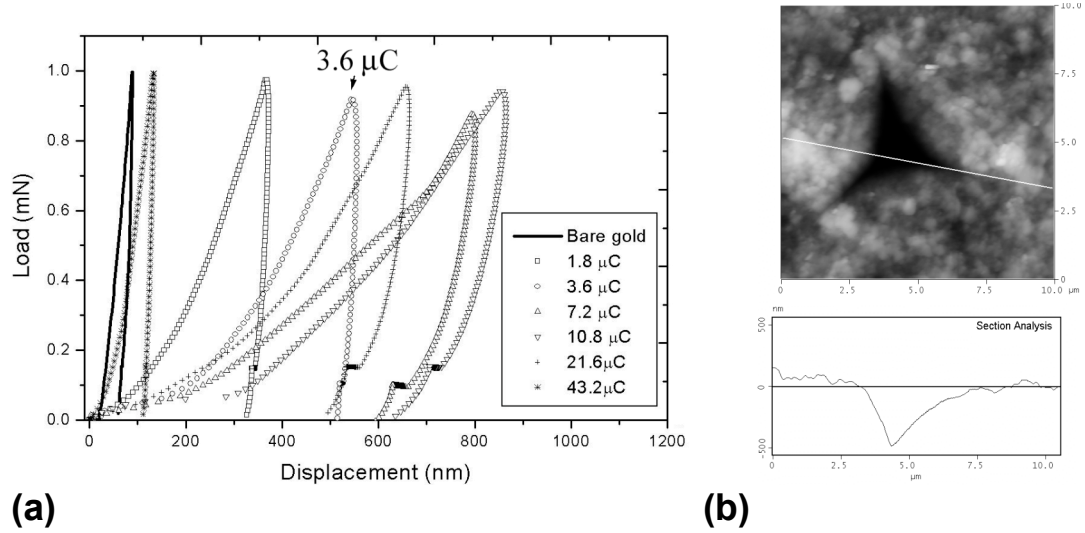


Fig.1 (a) Load vs. displacement curves for PEDOT coated sites with different deposition charge (thickness) and (b) AFM indent image and section analysis of selected one site (with passing through deposition charge of 3.6  $\mu\text{C}$ ).

We have found that the effective stiffness, hardness and electrical properties of PEDOT coatings varied with thickness and morphology. Our results reveal that for rough surfaces of electrochemically polymerized PEDOT the minimum impedance correlates well with the mechanical properties. As can be seen from Fig. 2, the lowest impedance films (solid circles) are those that have the softest, largest displacement (open circles), and have the most compliant mechanical response (lowest stiffness and hardness) (Fig. 3). Our nanoindentation studies confirm that the coatings first become softer and rougher and then stiffen and get more fully dense as the polymerization continues (become thicker). This is consistent with the interpretation that the high effective surface area of the films promotes the most facile charge transport.

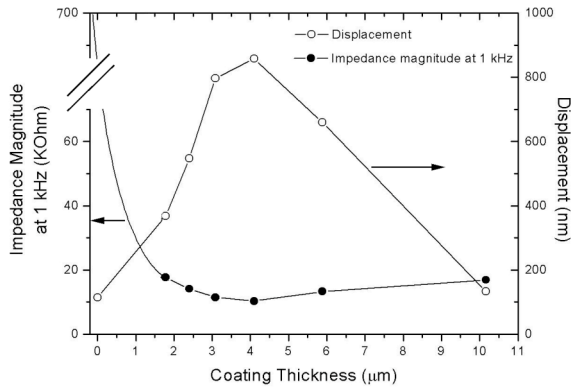


Fig.2 Impedance magnitude at 1 kHz and displacement as a function of coating thickness

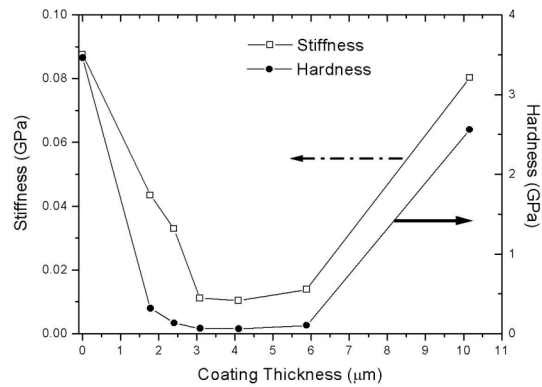


Fig.3 Elastic stiffness and plastic hardness vs. PEDOT coating thickness

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# **Incorporation of anti-inflammatory drug within electrospun biodegradable polymers for neural prostheses**

**Abstract-** We have demonstrated on incorporation of an anti-inflammatory drug (Dexamethasone) within electrospun poly(l,lactide) (PLLA) and poly(d,lactide) nanofibers. The degradation of biodegradable polymers of poly(l,lactide) (PLLA), poly(d,l lactide), poly(lactide-co-glycolide) (PLGA) 75/25 and 50/50 was studied by mass lost measurements during 10 days. The surface morphology of drug loaded nanofibers of PLLA was investigated by e;ectroscanning microscopy (SEM ).

## **1. Introduction**

Poly( $\alpha$ -hydroxy esters) such as poly(l,lactide) (PLLA), poly(d,l lactide) (PLDA), poly(lactide-co-glycolide) (PLGA) are bioresorbable polymers which are biocompatible and they can degraded by hydrolytic degradation. They can be widely used for biomedical applications such as tissue engineering<sup>1</sup>, drug delivery<sup>2</sup> and surgical sutures<sup>3</sup>. PLA has a slower degradation rate than PGA because of its hydrophobic methyl group in the polymer backbone. PGA is highly crystalline and is insoluble in many of organic solvents. In contract, amorphouse PLA (such as PLDA) can be dissolved in many common solvents. The combination of PLA and PGA can make more effective copolymers which has both chemical and physical properties. We have reported that electrospinning of poly( $\alpha$ -hydroxy esters) nanofibers will increase dramatically the ratio fo surface area to volume and decrease the density of polymers<sup>4</sup>. Therefore, the electrospun nanofibers are more flexible and less hydrophobic and suitable for wide range of biomedical applications. The degradation mechanism of bioresorbable polymers will be started by hydrolytic degradation of polymer chains and is due to the hydrolysis of ester group of polymer backbone<sup>5</sup>. This degradation is influenced by molecular weight, polymer composition ratio, ordered structures such as crystallinity, environmental condition and morphological variables such as porosity. Thus, we anticipate electrospun nanofibers which have diameter in the range of 20-800 nm have higher degradation rate in comparison with bulk of polymer. Dexamethasone is widely used in treatment of neurological disease as a anti-inflammatory drug to reduce cerebral edema associated with truma<sup>6</sup>. Because of limited ability to cross the blood-brain barrier, dexamethasone must be delivered in high systematic doses. to achieve therapeutic brain level. It has been shown that dexamethasone can be considered for neural prosthetic applications such as recording electrodes by loading in polymeric substrate<sup>7</sup>. In the present study, degradation rate of PLLA, PLDA, PLGA 75/25 and 50/50 has been monitored for 10 days and is still in progress. Also the dexamethasone has been loaded within electrospun PLLA with drug/polymer ratio of  $\frac{1}{2}$  and surface morphology of drug loaded nanofibers was investigated by scanning electron microscopy (SEM)..

## **2. Materials & Methods**

### **2.1 Materials**

The semi-crystalline PLLA, RESOMER<sup>®</sup> L 210, purchased from Boehringer Ingelheim Pharma GmbH & Co. KG (Germany) with inherent viscosity 3.3-4.3 dl/g, poly D,l-lactide, PLDA with inherent viscosity 0.5-0.65 dl/g (MEDISORB<sup>®</sup> 100DL Low IV) and poly lactid-co-glycolide, PLGA 75:25 (MEDISORB<sup>®</sup> 7525DL Low IV) with inherent viscosity 0.5-0.65 dl/g and PLGA 50:50 (MEDISORB<sup>®</sup> 5050DL High IV) with inherent viscosity 0.66-0.80 dl/g were purchased from Alkermes Inc. (OH, USA). Dexamethasone was obtained from ALEXIS<sup>®</sup> Biochemicals. Chloroform was prepared from Sigma.

## 2.2 Methods

PLLA, PLDA, PLGA 75/25 and PLGA 50/50 was dissolved in chloroform at 55 °C in the stirring condition to prepare a solution with the concentrations of %4 wt, %20 wt., %15 wt. and %15 wt. respectively. Also mixtures of PLLA and Dexamethasone with the ratio of 2/1 and 4/1 were prepared. Then chloroform was added to the PLLA/drug mixtures and they were placed in the stirring condition at 55°C for 24 hours in order to dissolve the PLLA. The polymer nanofibers were prepared from polymer solutions and polymer/drug solutions by electrospinning technique on the silicon substrate which were cut in 10x10 cm<sup>2</sup>. The electric field was 1 kV/cm with a distance of 10 cm between spinneret and the substrate and the solution feed rate was 0.25 ml/hr.

## 2.3 Degradation studies and morphological analysis

The samples of PLLA, PLDA, PLGA75/25 and 50/50 were weighted ( $g_0$ ) and then placed in closed petri dishes containing 15 ml of phosphate buffer solution (PBS, pH 7.27) and incubated in vitro at the temperature of  $37.0 \pm 0.1$  °C for different period of time. For the invitro degradation study, three specimens of each polymer nanofibers were recovered at the end of each degradation period. The samples were dried in vacuum oven at the room temperature for room temperature and were weighed ( $g_w$ ). The mass loss of the samples was calculated with the following equation based on the initial mass of each samples ( $g_0$ ) before incubation:

$$\%mass_{loss} = \frac{g_d - g_0}{g_0} \times 100$$

The degradation studies of samples for 10 days revealed that PLLA, PLDA and PLGA 75/25 have not yet tended to degrade but as it has been shown in figure 1. PLGA 50/50 nanofibers were completely degraded after 3 days. The morphology of dexamethasone loaded PLLA fibers was examined with Nova Focused Ion Beam (FIB) instrument in SEM mode and they has been shown in figures 2 and 3.

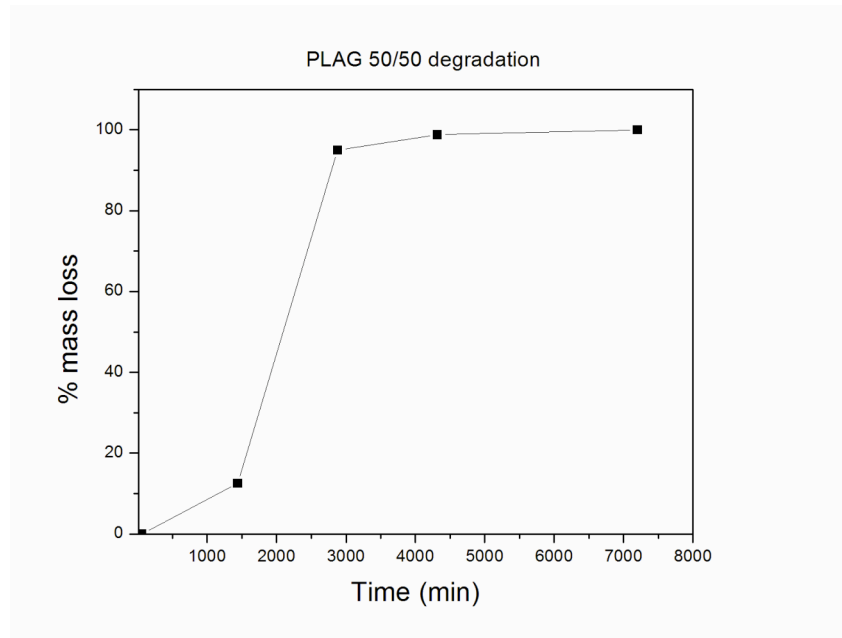


Fig. 1. Mass loss measurement of PLGA 50/50. The fibers completely dissolved after 3 days (4320 mins)

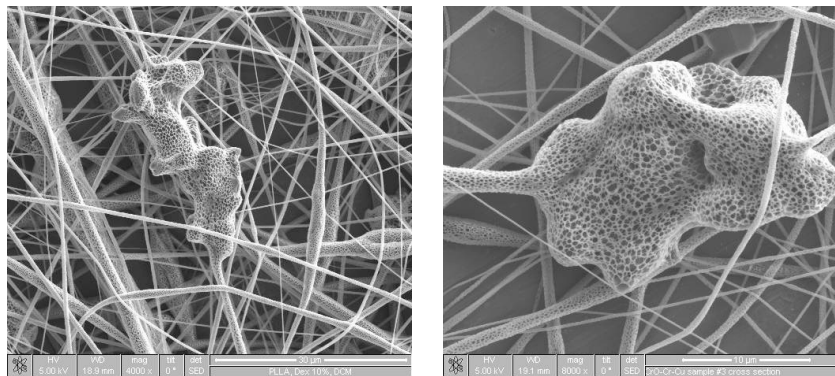


Fig. 2 SEM images of dexamethasone loaded PLLA fibers with concentration of 4/1 of polymer/drug.

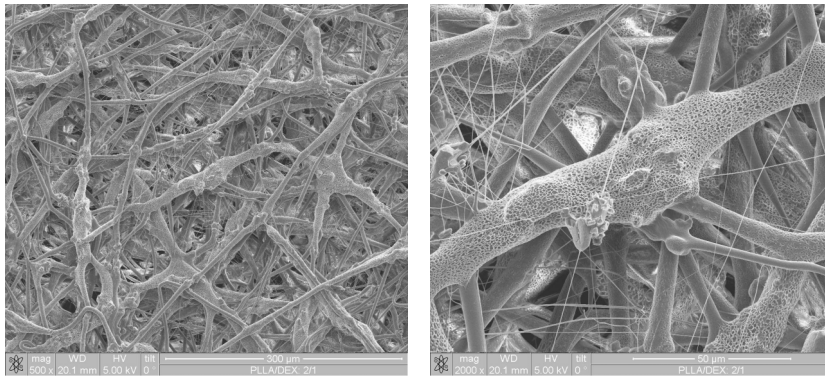


Fig. 2 SEM images of dexamethasone loaded PLLA fibers with concentration of 4/1 of polymer/drug.

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# Electrical and surface characterization of PEDOT species for neural electrodes

## AIM

The goal of this work is to understand the effects of biological molecular dopants and electrochemical processing parameters on the electrical and morphological properties of conductive poly(3,4-ethylenedioxy thiophene) (PEDOT) films for neural electrodes. Our lab has focused on methods for improving the biocompatibility of neural probe electrodes with neural cells for implanted long-term recording stability. In this study, we have investigated methods for producing sterile polymer films for use in vitro and in vivo, and for incorporating the cell adhesion peptide, poly-L-lysine (PLL) into the films. Other groups have looked at the incorporation of PLL onto microelectrode arrays by micro-contact printing (James et al., 2004), but we propose that incorporation of PLL into the bulk conductive polymer is possible by dip-coating of the electrode and subsequent electrochemical polymerization of PEDOT. We have also examined whether the ionic solvent choice for the monomer solution during electrochemical deposition has any effect on the formation, roughness, texture, impedance and charge capacity of these films.

## METHODS AND MATERIALS

### *Sterilization of EDOT monomer*

Sterilization of EDOT monomer was performed using a Tuttnauer 3870EA Autoclave or using 0.22µm Millex-GV filters. The control solution was used unsterilized.

### *Electrochemical Polymerization*

Samples were made in triplicate. Plastic coverslips were sputtered with approximately 1-3nm of platinum with a Technics Hummer VI Sputter Coater before polymerization. Electrochemical deposition of (3,4-ethylenedioxy thiophene) (EDOT) (BayerAM, Germany) to form conductive polymer films of PEDOT on plastic coverslips (Fisher) was performed using an AutoLab PGStat12 (EcoChimie, Netherlands). Electrochemical polymerization took place with galvanostatic current density of  $0.14\text{--}1.4 \times 10^{-5}$  A/mm<sup>2</sup> for 1, 5, 10, 30 minutes to produce films of varying thicknesses. Monomeric solutions of 0.01M EDOT were made in phosphate-buffered saline, pH 7.2 (EDOT/PBS), and in Hank's Balanced Salt Solution (EDOT/HBSS). PEDOT/PBS/PLL samples were soaked in a PLL solution for 15 mins at room temperature.

### *Electrical Characterization*

Electronic impedance spectroscopy (EIS) of the electrodes was performed at frequencies in the range of 1 Hz to 10<sup>5</sup> Hz using the Frequency Response Analyzer (FRA) program and the AutoLab PGStat12. For each type of solvent and sterilization method used, the mean of the impedances of the 3 samples were taken and compared to the other sample types.

Cyclic voltammetry (CV) was performed to quantify the charge capacity and capacitive behavior of the various PEDOT films. Using the General Purpose Electrochemical System



(GPES) program on a computer connected to the AutoLab PGStat12, the current of each electrode sample at various electromotive voltages was obtained over a range of -0.8V to 0.6V for 3 cycles.

### *Scanning Electron Microscopy*

After performing electrical characterization of the films, the samples were mounted on conductive stubs and were sputtered with a thin layer of platinum (<1nm) using a Technics Hummer VI Sputter Coater. A Phillips XL30 Field Emission Gun Scanning Electron Microscope (SEM) was used to visualize the surface topography, roughness, and texture.

## **RESULTS AND DISCUSSION**

Electropolymerization of all sterilized solutions yielded dark polymer films similar to the control unsterilized monomer solution when grown for 10 mins. The PEDOT film grown on top of PLL-coated electrodes also produced macroscopically similar films to the control. All polymer films had opaque dark blue/black appearance.

As seen in Figures 1a-c, electronic impedance spectroscopy revealed that at the biologically relevant frequency of 1 kHz, none of the films was remarkably different than the controls. The addition of PLL to the PEDOT film seemed to make the impedance more stable across the frequency spectrum. Autoclaving of the monomer solutions before polymerization tends to result in lowered impedance at frequencies between 1k-100kHz. Any effect due to solvent type was also small compared with the standard error of means for the impedance data.

The cyclic voltammetry of PEDOT films shows characteristic charging and discharging of the polymer as the voltage is ramped up and down (Figures 2a-c). The charge capacity (area between curves) for the polymer films remains roughly constant and shows no overall trends for the sterilized species of PEDOT. The incorporation of PLL into the PEDOT film does increase the charge capacity of the polymer compared to the undoped polymer.

Surface morphology of the different PEDOT/PBS species was also unremarkable between samples, as seen in Figure 3. The polymer films showed characteristic nodular texture, responsible in large part for increased surface area and reduced impedance of the films. The sterilized samples demonstrated that the autoclaving and filtering processes did not harm the integrity of the monomer, or its ability to polymerize into low impedance, conductive polymer films on metallic electrodes. Similar results were encountered for the PEDOT/HBSS species.

The sterilization of PEDOT films for neural electrode coatings is feasible without affecting the electrical or morphological properties of the films. The incorporation of adhesion peptide PLL did not affect the surface of the polymer, but might have some effect at stabilizing the impedance. This will be further investigated as will the effect of the adhesive dopant on cultured neurons.

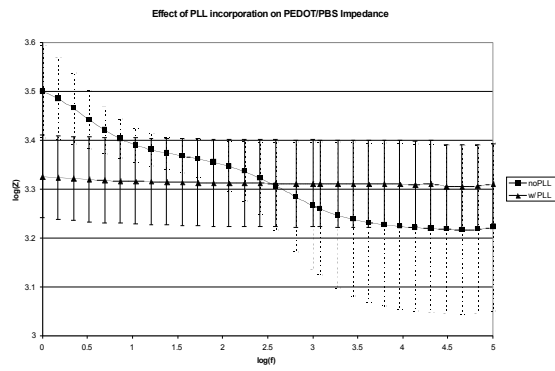
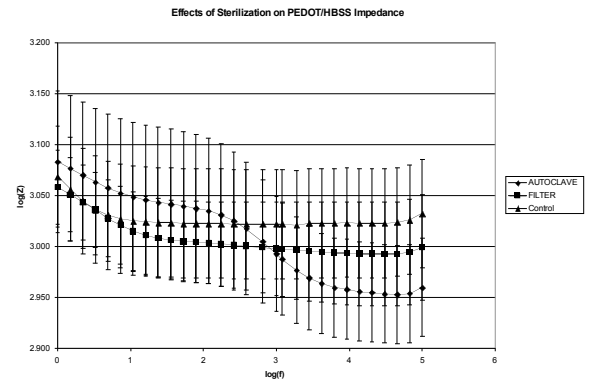
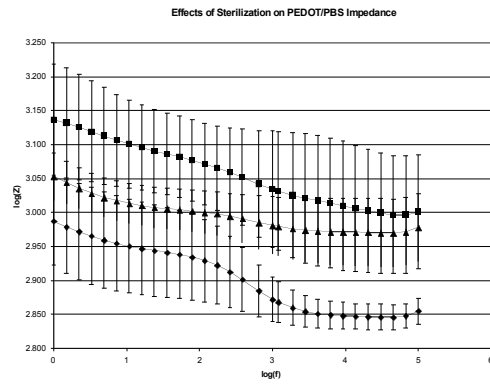


Figure 1a-c. Impedance spectra for top-left a) PEDOT/PBS, top-right b) PEDOT/HBSS, and bottom-left c) PEDOT/PBS/PLL

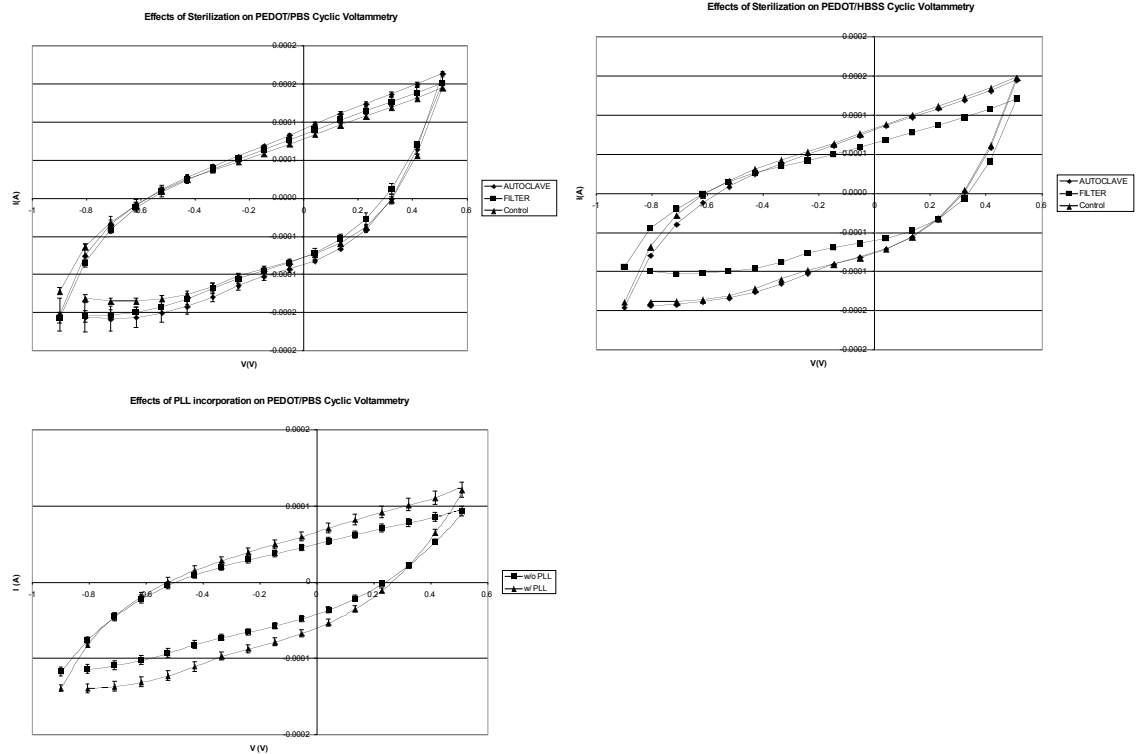
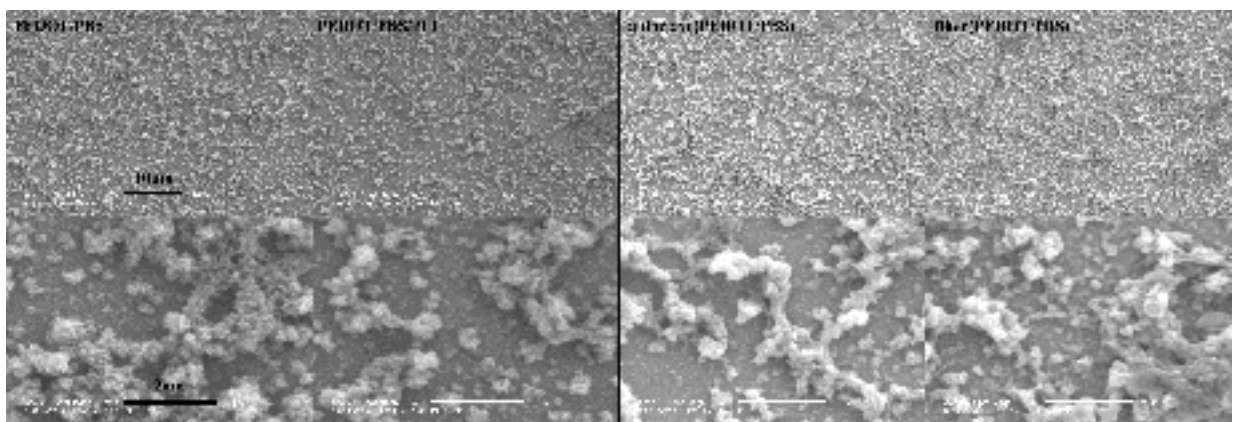
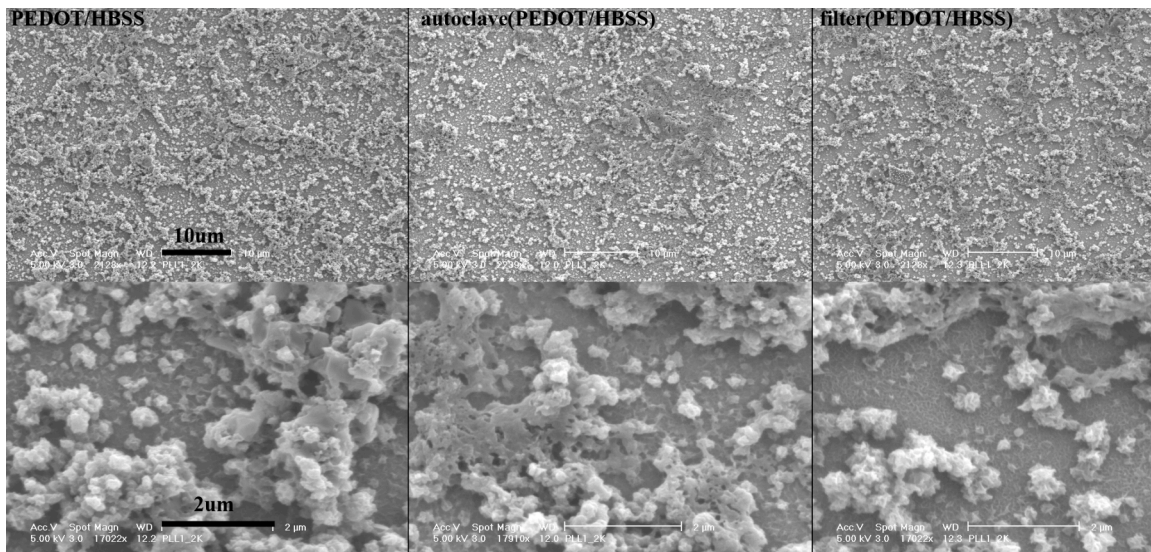


Figure 2a-c. Cyclic voltammetry for top-left a) PEDOT/PBS, top-right b) PEDOT/HBSS, and bottom-left c) PEDOT/PBS/PLL



Figures 3a (top),b(bottom). 3a) shows the PEDOT/PBS species' morphologies at 2k and 18k magnification using the SEM. 3b) shows the PEDOT/HBSS species' structures at similar magnifications. Scale bars are for entire rows.

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